1083-8791/06/1210-0001$32.00/0
doi:10.1016/j.bbmt.2006.06.007

Unmanipulated HLA 2–3 Antigen-Mismatched (Haploidentical) Stem Cell Transplantation Using Nonmyeloablative Conditioning

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Received March 19, 2006; accepted June 7, 2006

ABSTRACT

The major problems in human leukocyte antigen (HLA)-mismatched stem cell transplantation (SCT) are graft failure and graft-versus-host disease (GVHD). Less-intensive regimens should be associated with a lower release of inflammatory cytokines and possibly less GVHD. The objective of this study was to investigate whether HLA-haploidentical SCT can be performed using nonmyeloablative conditioning and pharmacologic GVHD prophylaxis, including glucocorticoids. Using conditioning consisting of fludarabine, busulfan, and anti–T-lymphocyte globulin and GVHD prophylaxis consisting of tacrolimus and methylprednisolone (1 mg/kg/day), 26 patients who had hematologic malignancies in an advanced stage or with a poor prognosis underwent transplantation using peripheral blood stem cells from an HLA-haploidentical donor (2–3 antigen mismatches in the graft-versus-host [GVH] direction) without T-cell depletion. All patients except for 1 achieved donor-type engraftment. Rapid hematologic engraftment was achieved (neutrophils > 0.5 × 10^9/L on day 12 and platelets > 20 × 10^9/L on day 12), with full donor chimerism achieved by day 14. Fifteen patients did not develop acute GVHD clinically, and only 5 patients developed grade II GVHD. The recovery of $CD4^+$ T cells was delayed compared with that of $CD8^+$ T cells. Sixteen of the 26 patients are alive in complete remission. Four died of transplantation-related causes, and 6 died of progressive disease. These data suggest that nonmyeloablative conditioning, GVHD prophylaxis consisting of tacrolimus and methylprednisolone, and early therapeutic intervention for the GVH reaction allow stable engraftment and effectively suppress GVHD in HLA 2–3 antigen-mismatched SCT.

KEY WORDS

Allogeneic stem cell transplantation • Nonmyeloablative stem cell transplantation • HLA-mismatched transplantation • Graft-versus-host disease

INTRODUCTION

Effective allogeneic stem cell transplantation (SCT) regimens have been established using human leukocyte antigen (HLA)-matched donors. However, > 70% of patients who could benefit from allogeneic SCT do not have a matched sibling donor. On the other hand, the likelihood of promptly identifying an HLA-haploidentical donor within the family is > 90%. In allogeneic SCT, 2 immunological barriers must be overcome: (1) the rejection barrier, or host-versus-graft (HVG) reaction, and (2) the graft-versus-host (GVH) reaction. Because the occurrence of rejection or severe acute GVH disease (GVHD) is reportedly associated with the degree of HLA incompatibility between the donor and recipient [1,2], both of these barriers must be more difficult to overcome in HLA-haploidentical transplantation setting than in HLA-matched settings.

One useful approach to overcoming these barriers is to use T-cell–depleted grafts. Since the 1990s, HLA 2–3 antigen-mismatched (haploidentical) SCT has been investigated by several groups, using partial T-cell depletion combined with intensive immunosup-

pression [3] or megadose $CD34^+$ cell transplantation [4,5]. These studies produced encouraging outcomes regarding the achievement of engraftment or suppression of GVHD. Furthermore, Aversa et al. [6] recently reported, in a phase II study treating 101 patients, good survival rates with excellent quality of life in patients with transplantation in remission.

On the other hand, nonmyeloablative stem cell transplantation (NST) from an HLA-matched donor has come to be generally accepted as a method of allogeneic SCT for patients considered ineligible for myeloablative preparative regimens because of advanced age or comorbidities. The total body irradiation (TBI) or high-dose cyclophosphamide (CPA) commonly used as conditioning treatments for conventional myeloablative SCT induces the production of inflammatory cytokines, such as tumor necrosis factor-α and interferon-γ, known to be deeply involved in the pathogenesis of GVHD [7]. Accordingly, the use of nonmyeloablative conditioning may have an advantage in reducing the magnitude of the GVH reaction after HLA-haploidentical SCT. However, to date, there have been only a few reports on studies of NST from HLA-haploidentical donors [8–10]. To achieve bidirectional (GVH and HVG) tolerance, Sykes et al. used a nonmyeloablative conditioning consisting of high-dose CPA, thymic irradiation, and anti–T-lymphocyte globulin (ATG) [8] or anti-CD2 monoclonal antibody [9]. Furthermore, O'Donnell et al. [10] concluded that using high-dose CPA early after bone marrow transplantation attenuated both the GVH and HVG reactions, and consequently used a nonmyeloablative regimen including fludarabine, 2 Gy of TBI, and posttransplantation CPA. The 2 foregoing approaches were found to produce long-lasting mixed chimerism over the HLA barrier (although further methodological improvement is needed to suppress severe GVHD or graft rejection).

We chose a conditioning regimen comprising fludarabine, busulfan, and ATG to avoid the use of high-dose CPA, which is associated with organ toxicities (eg, myocardial damage). This conditioning regimen was originally established by Slavin et al. [11] for HLA-identical NST. We recently conducted a pilot study of HLA-haploidentical NST using this conditioning regimen [12] and found that stable, rapid donor engraftment was achieved; all of the patients achieved almost 100% donor chimerism of both T-cell and myeloid cell lineages by day 14. However, cyclosporine (CSP) or tacrolimus (FK506) monotherapy, the main GVH prophylaxis in that study, was insufficient for controlling GVHD. In particular, during the engraftment period, some patients developed very aggressive acute GVHD. These patients first had a high fever for 2–3 days, with increased serum-soluble interleukin-2 receptor (sIL-2R) levels, followed by the development of GVHD symptoms, such as skin eruption (unpublished data). Methylprednisolone (mPSL) 2 mg/kg was administered to control the GVHD symptoms with little effect; these patients ultimately developed fatal thrombotic microangiopathy (TMA) secondary to severe GVHD. Even intense immunosuppressive treatment has little effect once GVHD is fully established even in HLA-matched SCT [13]. Consequently, many investigators agree that GVHD should be prevented rather than treated.

Based on these findings, in the present NST protocol using HLA-haploidentical donors, we increased the ATG dose and intensified the GVHD prophylaxis (using the combination of FK506 + mPSL 1 mg/kg). Furthermore, we planned to perform early therapeutic interventions for signs or symptoms of GVH in the engraftment period (ie, high fever, increased sIL-2R level). We performed transplantation in 26 patients with hematologic malignancies in an advanced stage or with a poor prognosis using peripheral blood stem cells (PBSCs) from a related donor with HLA 2–3 antigen mismatches (haploidentical) in the GVH direction, and assessed the donor engraftment rate and the incidence and severity of GVHD. We found that this treatment regimen allowed stable engraftment and effectively suppressed GVHD after HLA-haploidentical SCT.

PATIENTS AND METHODS

Patients

The major objective of this study was to determine the donor engraftment rate on day 35 after transplantation, as well as the incidence and severity of acute GVHD. A total of 26 consecutive adult patients who had hematologic malignancies in an advanced stage or with a poor prognosis underwent SCT with a reduced-intensity preconditioning treatment using non–T-cell–depleted PBSCs from an HLA-haploidentical related donor (2–3 antigen mismatches in the GVH direction) at Osaka University Hospital. The patient group comprised adult patients age 40 years or older, along with younger patients considered ineligible for myeloablative preparative regimens because of comorbidities. The patients either lacked an available HLA-identical related or HLA-phenotypically identical unrelated donor, or urgently needed a transplant. The patient characteristics are given in Table 1. The clinical courses of 3 of the 26 patients have been detailed in previous reports [14–16]. More than half of the patients were chemoresistant at the time of transplantation. All 3 of the patients who underwent transplantation in the first complete remission (CR) had Philadelphia chromosome–positive acute lymphoid leukemia. Four patients had a previous autologous SCT, and all of them underwent HLA-haploidentical

Table 1. *Patient Characteristics*

Patient	UPN	Disease	Sex	Age	Disease status	Donor	Sex/age (donor)	CD34 cells ($\times 10^6$/kg)	CD3 cells ($\times 10^8$/kg)	CD56 cells ($\times 10^7$/kg)
1	218	CML	M	50	CP2	Sibling (M)	F/54	3.03	1.38	1.91
2	227	AML	M	53	CR2	Daughter	F/26	4.30	0.52	1.22
3	109	MDS	M	42	Overt	Sibling (P)	M/38	7.50	3.59	8.10
4	246	MDS	M	29	Overt	Sibling (M)	M/29	12.30	2.54	10.64
5	266	AML	M	41	CR2	Sibling (P)	M/34	7.40	2.22	3.13
6	269	NHL	F	27	Re*	Sibling (M)	F/26	22.00	5.71	10.76
7	286	NHL	M	45	Re*	Son	M/19	7.92	2.79	6.56
8	288	AtCML	M	53	CP	Son	M/17	5.60	2.10	2.62
9	292	MDS	M	54	Overt	Daughter	F/27	4.00	3.35	5.30
10	297	AML	M	52	CR2	Son	M/16	4.08	5.97	11.10
11	299	NHL	M	57	RR	Son	M/25	7.75	5.44	6.44
12	302	AML	F	44	CR2	Son	M/17	5.20	2.76	5.35
13	304	ALL	F	45	CR1(Ph)	Daughter	F/14	9.00	1.56	1.99
14	306	MDS	M	47	Overt	Son	M/17	3.90	NT	NT
15	308	My/NK	M	50	RR	Son	M/22	6.20	1.71	3.31
16	310	ALL	M	57	CR1(Ph)	Daughter	F/28	11.00	2.50	3.40
17	315	ATL	M	52	RR	Son	M/24	6.90	1.33	3.27
18	316	NHL	F	38	Re*	Daughter	F/14	5.10	2.48	6.15
19	317	NHL	F	49	RR	Son	M/20	4.30	1.99	2.61
20	249	NHL	M	54	Re*	Sibling (M)	M/49	6.13	0.84	2.07
21	322	MDS	F	59	Overt	Daughter	F/33	7.43	2.04	4.56
22	324	AML	M	39	PR	Sibling (U)	F/37	9.80	3.68	4.25
23	328	AML	F	46	RR	Son	M/20	2.45	2.73	4.05
24	329	ALL	F	51	CR1(Ph)	Son	M/23	8.50	3.07	3.36
25	333	NHL	F	47	RR	Daughter	F/19	5.20	3.24	5.69
26	335	AML	F	59	RR	Daughter	F/31	8.78	3.21	6.41

AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; CML, chronic myeloid leukemia; AtCML, atypical CML, MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; My/NK, myeloid natural killer cell leukemia; ATL, adult T-cell leukemia; CR1, first complete remission; CR2, second complete remission; PR, partial remission; Re, relapse; RR, resistant relapse; CP, chronic phase; CP2, second CP; overt, transformation from MDS to acute leukemia; NT, not tested.

All of the patients with ALL who underwent allogeneic NST in CR1 had the Ph1 chromosome. Sibling (P) and sibling (M) indicate noninherited paternal antigen (NIPA)-mismatched sibling and noninherited maternal antigen (NIMA)-mismatched sibling, respectively. Sibling (U) indicates that whether the donor was an NIMA- or NIPA-mismatched sibling was not determined.

*The patient had a relapse after autologous peripheral stem cell transplantation.

NST due to recurrence of the original disease. Donors and patients shared 1 HLA haplotype, but the other haplotype differed. The HLA disparities between donors and recipients are shown in Table 2. Fourteen and 12 donors had 4/6 and 3/6 matches in the GVH direction, respectively; 4, 13, and 9 donors had 5/6, 4/6, and 3/6 matches in the HVG direction, respectively. Seven transplantations were performed using grafts from sibling donors; 4 patients received grafts from noninherited maternal antigen [17] (NIMA)-mismatched donors, 2 received grafts from noninherited paternal antigen (NIPA)-mismatched donors, and the relevant status could not be examined for 1 donor–recipient pair. Nineteen transplantations were performed using grafts from offspring donors (11 sons and 8 daughters). The median patient age was 49.5 years (range, 27–59 years).

Institutional review board approval was obtained for the treatment protocol, and written informed consent was obtained from the patients and their families.

Preparative Regimen for Transplantation

Before conditioning, 13 patients who had progressive disease at the time of transplantation received chemotherapy for tumor reduction. Eight patients with myeloid leukemia received continuous intravenous cytarabine 60 mg/m^2/day for varying periods (6–14 days) depending on the tumor burden. Five patients with B-cell lymphoma received rituximab 600 mg twice.

The conditioning regimen comprised fludarabine 30 mg/m^2/day for 6 days (day −10 to day −5), oral busulfan 4.0 mg/kg/day for 2 days (day −6 to day −5), and rabbit ATG (Fresenius, Munich, Germany) 2 mg/kg/day for 4 days (day −4 to day −1). To prevent the anaphylaxis caused by ATG, mPSL was administered at 1 mg/kg on days −4 to −1 and thereafter continued as GVHD prophylaxis at the same dose. PBSC mobilization using granulocyte colony-stimulating factor (G-CSF) was performed as described previously [18]. Two leukapheresis procedures were performed on days 4 and 5 of G-CSF administration. After leuka-

Table 2. *HLA Disparities Between Donors and Recipients*

Patient	UPN	Recipient*	Donor	No. of mismatched antigens†	
				GVH	HVG
1	218	A26B61DR4/A24B52DR12	A26B60DR12/A24B52DR12	2(B, DR)	1(B)
2	227	A24B51DR4/A11B46DR8	A24B52DR9/A11B46DR8	2(B, DR)	2(B, DR)
3	109	A24B35DR8/A24B15DR4	A2B13DR12/A24B15DR4	2(B, DR)	3
4	246	A24B7DR8/A31B54DR15	A2B13DR12/A31B54DR15	3	3
5	266	A11B62DR14/A24B61DR12	A2B35DR8/A24B61DR12	3	3
6	269	A24B61DR9/A24B48DR4	A2B54DR4/A24B48DR4	2(B, DR)	2(A, B)
7	286	A24B46DR8/A31B61DR9	A2B61DR4/A31B61DR9	3	2(A, DR)
8	288	A2B55DR9/A24B52DR15	A24B7DR1/A24B52DR15	3	2(B, DR)
9	292	A24B52DR15/A24B35DR9	A11B61DR16/A24B35DR9	2(B, DR)	3
10	297	A24B52DR2/A2B62DR4	A2B46DR9/A2B62DR4	3	2(B, DR)
11	299	A24B48DR14/A2B51DR9	A26B61DR12/A2B51DR9	3	3
12	302	A24B54DR1/A11B70DR4	A24B51DR11/A11B70DR4	2(B, DR)	2(B, DR)
13	304	A31B51DR14/A11B35DR15	A2B46DR4/A11B35DR15	3	3
14	306	A11B51DR4/A24B39DR8	A24B54DR4/A24B39DR8	2(A, B)	1(B)
15	308	A24B7DR1/A31B54DR4	A24B60DR11/A31B54DR4	2(B, DR)	2(B, DR)
16	310	A24B52DR16/A26B35DR8	A24B61DR9/A26B35DR8	2(B, DR)	2(B, DR)
17	315	A11B62DR9/A24B52DR15	A2B52DR9/A24B52DR15	2(A, B)	1(A)
18	316	A24B62DR9/A2B60DR12	A2B46DR8/A2B60DR12	3	2(B, DR)
19	317	A24B62DR9/A2B39DR12	A2B62DR4/A2B39DR12	2(A, DR)	1(DR)
20	249	A2B60DR4/A24B61DR9	A24B52DR15/A24B61DR9	3	2(B, DR)
21	322	A2B46DR8/A24B7DR1	A2B35DR12/A24B7DR1	2(B, DR)	2(B, DR)
22	324	A11B67DR9/A2B62DR2	A11B75DR4/A2B62DR2	2(B, DR)	2(B, DR)
23	328	A2B71DR8/A24B52DR9	A2B61DR4/A24B52DR9	2(B, DR)	2(B, DR)
24	329	A26B39DR11/A24B61DR15	A3B44DR13/A24B61DR15	3	3
25	333	A2B35DR8/A26B51DR14	A33B61DR4/A26B51DR14	3	3
26	335	A26B39DR15/A2B62DR12	A31B51DR8/A2B62DR12	3	3

*The HLA haplotype before or after a slash is one that is not or is shared between donors and recipients, respectively. HLA antigens that are underlined in recipients or in donors denote mismatched target antigens in GVH or HVG reactions, respectively.

†The locus(loci) of the mismatches are given in parentheses for cases involving fewer than all loci.

pheresis, PBSCs were infused on days 0 (the day of leukapheresis) and +1 without T-cell depletion. Patients received G-CSF if absolute neutrophil count (ANC) dropped to $< 0.5 \times 10^9$/L after transplantation.

GVHD Prophylaxis and Treatment

FK506 treatment was initiated on the day before transplantation, given at 0.02 mg/kg/day in a continuous infusion. Intravenous administration of mPSL was started at a dose of 1 mg/kg/day from day −4 as described earlier. The target blood concentration of FK506 was set at 10–15 ng/mL up to day 30, and thereafter tapered in the absence of acute GVHD. Patients received intravenous FK506 therapy until they could reliably receive oral medications after transplantation. At that time, they were switched to an oral dose calculated to be 4 times their current intravenous dose. The oral dose was administered in 4 divided doses every 6 h. The dose reduction of mPSL was started on day 15 and done relatively rapidly until day 30 (the target dose on day 30 was 0.5 mg/kg/day) using the serum sIL-2R level [19,20] as an indicator, and carefully continued thereafter. At a dose of 0.5 mg/kg, mPSL was switched to an oral prednisone formulation (PSL).

Acute GVHD was graded according to standard criteria [21]. Diagnosis and grading of chronic GVHD followed the Seattle criteria [22]. The severity of disease was also assessed by the Karnofsky performance rating. When GVHD (≥ grade I) was diagnosed, the steroid dose was increased (mPSL 2 mg/kg/day at the maximum dose) depending on the severity of GVHD, and mycophenolate mofetil (MMF) was started at a dose of 15 mg/kg/day. When GVHD was resistant to these treatments, ATG was administered to patients with a CD3 cell count > 500/μL, and antitumor necrosis factor-α antibody (infliximab) 10 mg/kg was administered to the other patients. Patients who developed high-grade fever (above 38°C) while negative (or low-grade positive) for C-reactive protein during the engraftment period, with obvious evidence for bacterial, fungal, or viral infections, were diagnosed as having GVH reaction and given infliximab 10 mg/kg with the start of MMF 15 mg/kg/day. In patients who developed TMA, the doses of FK506 and mPSL were tapered rapidly, and MMF 750–1000 mg/day was started. When the original malignant disease relapsed

or regrew in the absence of GVHD, tapering of the FK506 and steroid doses was sped up, depending on the patient's disease status.

The sIL-2R level was monitored to estimate the degree of GVH response [19,20]. The normal sIL-2R level is < 570 U/mL.

Supportive Care

Each patient was isolated in a laminar air-flow room, and standard decontamination procedures were followed. Oral antibiotics (eg, ciprofloxacin, vancomycin, amphotericin B) were administered to sterilize the bowel. Patients with negative cytomegalovirus (CMV) IgG titers received blood products from CMV-seronegative donors. Intravenous immunoglobulin was administered at a minimum dose of 100 mg/kg every 2 weeks until day 100. Cotrimoxazole was given for at least 1 year for prophylaxis of *Pneumocystis carinii* infections. Acyclovir was administered at 1000 mg/day for 5 weeks after transplantation to prevent herpes simplex infections, then continued at 200 mg/day for at least 2 years thereafter.

Ganciclovir 7.5 mg/kg/day in 3 divided doses was administered from day -10 to day -3 as prophylaxis for CMV infection. Detection of CMV antigenemia was performed using an immunoperoxidase-conjugated monoclonal antibody, *HRP-C7*, which binds an immediate–early antigen of CMV [23], pp65 antigen. The degree of antigenemia was expressed as the number of CMV antigen–positive cells per 50,000 leukocytes. pp65 antigen testing in peripheral blood was performed once weekly. After grafting, ganciclovir administration was reinstituted in patients demonstrating positive CMV antigenemia.

TMA was diagnosed according to Zeigler's criteria [24] and based on recent recommendations reported by Nishida et al. [25].

Chimerism Analysis

After transplantation, serial blood samples were analyzed to determine the degree of donor/recipient chimerism in the T-cell– or neutrophil-enriched cell fraction, using polymerase chain reaction amplification of informative microsatellite regions to identify differences between donor and recipient (based on polymorphisms found in pretransplantation donor/recipient samples) [12]. To remove monocytes, KAC-2 silica beads (Japan Immunoresearch Laboratories, Gunma, Japan) were mixed with heparinized peripheral blood and incubated at 37°C for 1 h. To enrich T cells, a negative selection system (RosetteSep; StemCell Technologies, Vancouver, Canada) was used [26]. To obtain a T-cell–enriched cell fraction, a cocktail containing anti-CD16, anti-CD19, anti-CD36, and anti-CD56 antibodies was added to the blood samples after they were treated with the silica beads. After Ficoll-Paque (Pharmacia, Uppsala, Sweden) density-gradient centrifugation, $CD3^+$ cells with a purity of $> 95\%$ were recovered from the Ficoll–plasma interface. Neutrophils with a purity of $> 99\%$ were recovered from the Ficoll–red blood cell interface.

Statistical Methods

Statistical analyses were performed with SPSS software, version 11.0 (SPSS, Chicago, IL). Results were considered statistically significant when P was $\leq .05$. Estimates of the incidence of acute GVHD and of the probability of relapse were calculated using the Kaplan-Meier method. Comparison of the incidence of grade II acute GVHD between HLA 2 and 3 antigen-mismatched NSTs was performed based on the results of log-rank tests. In the analysis of event-free survival, rejection, relapse, death, and retransplantation were defined as events. Estimates of the probability of event-free survival were calculated using the Kaplan-Meier method.

RESULTS

Engraftment

Patients received PBSCs containing a median of 6.55×10^6 (range, $2.45–22.0 \times 10^6$) $CD34^+$ cells/kg without T-cell depletion. Consequently, a median of 2.54×10^8 (range, $0.52–5.97 \times 10^8$) $CD3^+$ cells/kg and a median of 4.25×10^7 (range, $1.22–11.10 \times 10^7$) $CD56^+$ cells/kg were transfused. All but 1 patient (UPN 246) achieved donor-type hematopoietic engraftment (Table 3; Figure 1). In 6 patients (24.0%), ANC did not drop below 0.1×10^9/L, and for the entire group it took a median of 8 days for ANC to drop below 0.1×10^9/L (Figure 1A). The median duration of ANC $< 0.1 \times 10^9$/L was 2 days (range, 0–13 days). Three patients (UPN 315, UPN 328, and UPN 329) never exhibited ANC $< 0.5 \times 10^9$/L. The remaining 22 patients achieved neutrophil engraftment (ANC $> 0.5 \times 10^9$/L) at a median of 12 days (range, 9–16 days) after transplantation (Figure 1B). Among the 25 patients who achieved donor-type hematopoietic engraftment, 1 patient (UPN 306) had no platelet recovery due to relapse. It took a median of 7 days (range, 2–15 days) for the platelets to drop below 20×10^9/L (Figure 1C). The platelet count never dropped below 20×10^9/L in 3 patients (UPN 315, UPN 328, and UPN 333), and these patients had no need for platelet transfusion. The remaining 22 patients achieved an unsupported platelet count of 20×10^9/L at a median of 12 days (range, 9–28 days) (Figure 1D).

GVHD

Fifteen (60.0%) of the 25 patients who achieved donor-type engraftment did not develop acute GVHD

Table 3. *Outcome*

Patient	UPN	Disease	Engraftment		GVHD		Survival days	Performance Status(%)	Summary of outcome
			Neu*	Plt†	acute	chronic			
1	218	CML	12	12	0	NA	62	—	CR→adenovirus infection
2	227	AML	13	16	0	Lim-S	>1697	100	CR
3	109	MDS	13	27	0	NA	39	—	CR→pulmonary fungal infection
4	246	MDS	−	−	NA	NA	>1438	100	rejection→2nd NST→CR
5	266	AML	14	11	II‡	Ext-S	638	—	CR→Rel→PD
6	269	NHL	12	9	0	—	>1159	100	CR
7	286	NHL	10	20	0	—	>907	100	CR
8	288	AtCML	14	28	0	Lim-S	>857	100	CR
9	292	MDS	11	14	I	Ext-S	>810	100	CR
10	297	AML	15	16	0	Ext-S	>720	100	CR
11	299	NHL	10	10	0	—	>696	100	CR
12	302	AML	14	12	0	Lim-S	>664	100	CR
13	304	ALL	11	17	II	Ext-S	220	—	CR→Rel→PD
14	306	MDS	16	−	I	NE	165	—	CR→Rel→PD
15	308	My/NK	14	9	II	—	255	—	CR→Rel→PD
16	310	ALL	9	9	I	—	>566	90	CR
17	315	ATL	+	+	II‡	Ext-S,E,O	382	—	CR→Rel→PD
18	316	NHL	11	10	0	Lim-S	>471	100	CR
19	317	NHL	12	19	0	—	120	—	PD
20	249	NHL	12	11	0	—	>355	100	CR
21	322	MDS	11	18	0	NA	30	—	CR→TMA
22	324	AML	9	10	0	—	140	—	CR→HC→TMA
23	328	AML	+	+	I	—	>209	100	CR
24	329	ALL	+	11	II	—	>195	100	CR
25	333	NHL	14	+	I	—	>118	100	CR
26	335	AML	11	14	0	NE	>69	100	CR

NA, not applicable; NE, not evaluable; Rel, relapse; PD, progressive disease; TMA, thrombotic microangiopathy; HC, hemorrhagic cystitis; Lim, limited GVHD; Ext, extensive GVHD. GVHD organ involvement: E, eyes; O, oral; S, skin.

*First day when ANC rose above 0.5×10^9/L.

†First day when platelet count rose above 20×10^9/L; transfusion-independent.

‡The 2 patients (UPN266 and UPN315) developed acute GVHD after the rapid tapering off of steroid and DLI because of lymphoproliferative disease and severe hemorrhagic cystitis, respectively.

+, Indicates that neutrophil and platelet count did not decrease to $<0.5 \times 10^9$/L and 20×10^9/L, respectively.

−, Indicates that neutrophil and platelet count did not recover to $>0.5 \times 10^9$/L and 20×10^9/L, respectively.

clinically (Table 3). Five patients (20.0%) developed grade II acute GVHD (Figure 2). One of these patients (UPN 315) developed cutaneous GVHD on day 39 after the rapid tapering of steroids and infusion of donor lymphocytes (containing 1×10^6 $CD3^+$ cells/kg) for TMA and hemorrhagic cystitis. Another patient (UPN 266) also developed grade II cutaneous GVHD on day 94 after the tapering off of steroids and infusion of donor lymphocytes for lymphoproliferative disease. All patients with grade II GVHD except 1 (UPN 308, who developed intestinal GVHD) exhibited only skin lesions. In terms of the impact of an HLA disparity in the GVH direction on the occurrence of grade II GVHD, the incidence of acute GVHD was 14.3 % (2/14) for HLA 2 antigen-mismatched SCT and 27.3 % (3/11) for HLA 3 antigen-mismatched SCT. There was no significant difference in the occurrence of acute GVHD between the 2 groups ($P = .615$; log-rank test). The treatment for GVHD was performed as described in Patients and Methods. Two patients (UPN 297 and UPN 299) had a high-grade fever at the time of engraftment, which was considered to indicate strong GVH reaction. Both patients received infliximab and MMF (see Patients and Methods), and neither patient developed acute GVHD clinically. In all of 5 patients with grade I GVHD and in 2 of 5 patients with grade II GVHD, the GVHD was successfully controlled by increasing the steroid dose and starting MMF. The remaining 3 patients with grade II GVHD also needed infliximab or ATG to suppress the GVHD. None of these 3 patients died of uncontrollable acute GVHD.

Of the 20 patients who could be evaluated for chronic GVHD, 5 (25%) developed an extensive form of chronic GVHD. All of these 5 patients had skin lesions, and 1 also had eye and mouth lesions. In these cases, chronic GVHD was controlled by increasing the doses of immunosuppressive agents. Although the tapering off of immunosuppressive agents or steroids was done successfully in only 1 patient, those patients who survived for more than 1 year exhibited relatively good performance status (90%–100% Karnofsky performance score) under low doses of immunosuppressive agents (FK506 1–3 mg/day; PSL 5–15 mg/day).

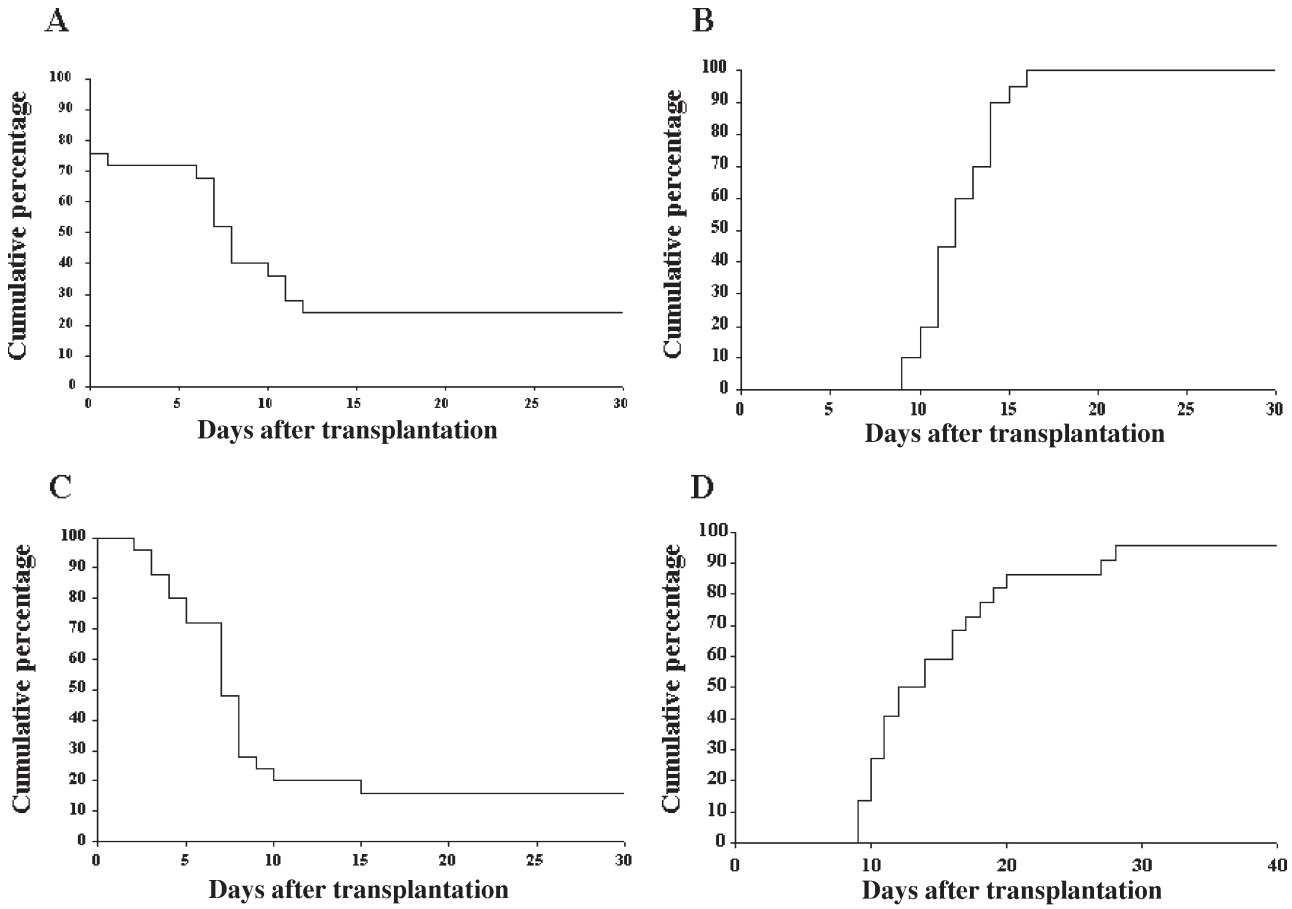

Figure 1. Engraftment of HLA-haploidentical stem cell allografts. Cumulative percentage of patients who never achieved ANC $< 0.1 \times 10^9$/L (A) and of patients who achieved ANC $> 0.5 \times 10^9$/L (B) is shown. The data for 3 patients in whom ANC did not decrease to $< 0.5 \times 10^9$/L were removed in (B). Cumulative percentage of patients in whom platelet counts never went below 20×10^9/L (C), and of patients in whom platelet counts recovered to $> 20 \times 10^9$/L (D) is shown. The data for 3 patients in whom platelet counts did not decrease to $< 20 \times 10^9$/L were removed in (D).

Chimerism Analysis

In all patients, chimerism status was monitored through a quantitative polymerase chain reaction method using informative microsatellite regions. In all of the 25 patients who achieved donor-type hematopoietic engraftment, 95%–100% donor chimerism was observed in T-cell and myeloid lineages by 2 weeks after transplantation (data not shown). The kinetics of engraftment of donor hematopoietic cells during the first 2 weeks after transplantation could be analyzed in 8 patients. As shown in Figure 3, donor T-cell engraftment was rapidly achieved; the median

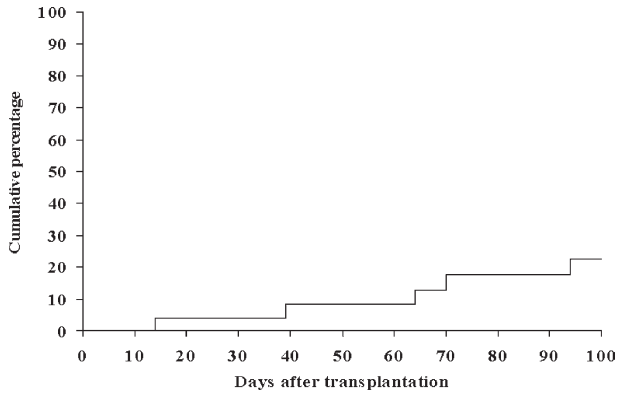

Figure 2. Cumulative incidence of acute GVHD (grade II).

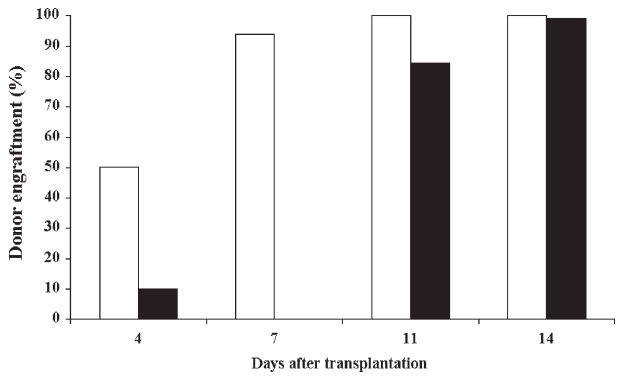

Figure 3. Chimerism status during the early stage of transplantation. Open and closed bars indicate a median percentage of donor $CD3^+$ cells and neutrophils, respectively. Data from 8 patients were analyzed.

value of donor T-cell components was 50.2% on day 4, 93.9% on day 7, and 100% on day 11. Donor neutrophil engraftment was slightly delayed compared with T-cell engraftment; the median value was 10% on day 4, 0% on day 7, 84.4% on day 11, and 100% on day 14. In most patients, neutrophils were of host origin on day 7, and rapidly changed to donor origin from host origin between days 7 and 14.

Lymphocyte Recovery and CMV Antigenemia

The $CD4^+$ T-cell count in the transplant cohort in this study recovered slowly, with the median cell count exceeding 100×10^6/L at 9 months after transplantation and 250×10^6/L at 18 months (Figure 4A). In contrast, the $CD8^+$ T-cell count recovered more rapidly (Figure 4B).

CMV antigenemia occurred in 21 of 26 patients (80.8%). The median peak number of CMV antigen–positive leukocytes was 11.1 per 50,000 white blood cells [WBCs] (range, 0.8/50,000–128.7/50,000). The median time to the maximum number of CMV antigen–positive leukocytes was day 35 (range, day 11–day 151). One patient (UPN 324) developed CMV enterocolitis, which was successfully controlled with foscarnet. All CMV antigenemia was controlled by the administration of ganciclovir and/or foscarnet.

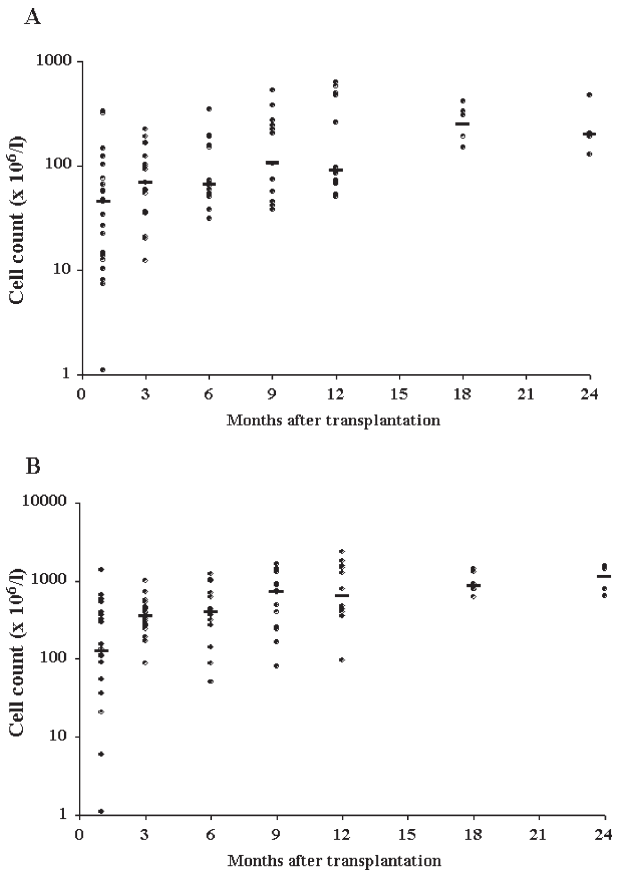

Figure 4. Lymphocyte recovery after HLA 2–3 antigen-mismatched NST. (A) $CD4^+$ cells. (B) $CD8^+$ cells. Horizontal bars indicate median values.

Table 4. *Adverse Events (%)*

Infections	
bacteria	
bacteremia	**0 (0)**
others	**2 (7.7)**
fungus	**1 (3.8)**
virus	
cytomegalovirus	**1 (3.8)**
others	**2 (7.7)**
Pneumocystis carinii	**0 (0)**
Hemorrhagic cystitis	**11 (42.3)**
Lymphoproliferative disease	**1 (3.8)**
FK506 encephalopathy	**0 (0)**
Thrombotic microangiopathy	**2 (7.7)**
Venoocclusive disease	**0 (0)**
Liver dysfunction*	**13 (50.0)**
PCI	**1 (3.8)**
Paralytic ileus	**2 (7.7)**
Nephrotoxicity†	**0 (0)**
Hyperglycemia‡	**12 (46.2)**
Hypertension	**4 (15.4)**
Arrhythmia	**1 (3.8)**
Venous thrombosis	**2 (7.7)**
Aseptic necrosis	**1 (3.8)**
Bone fracture§	**1 (3.8)**

PCI, pneumatosis cystides intestinalis.
*An increase to >3 times the normal upper limit of transaminase level.
†Nephrotoxicity requiring hemodialysis.
‡Insulin dose of >30 U/day was needed to control blood sugar.
§Compression fracture of vertebral body.

Other Complications

One patient (UPN 109) developed fatal fungal pneumonia during the neutropenic period (Table 4). Two patients had bacterial infections (pneumonia and cholecystitis), which were successfully treated with appropriate antibiotics. Eleven patients (42.3%) developed hemorrhagic cystitis. In 8 cases, the causal virus was detected: 3 cases of adenovirus, 4 cases of BK virus, and 1 case of BK virus and JC virus . One patient (UPN 218) developed fatal adenovirus pneumonia after hemorrhagic cystitis due to adenovirus. In other patients, hemorrhagic cystitis was improved by conservative therapy (hydration and/or bladder irrigation) with or without donor leukocyte infusion (DLI). Thirteen patients (50.0%) developed liver dysfunction with an increase to > 3 times the normal upper transaminase limit. Most cases of liver dysfunction were due to steroid- or drug-induced toxicities, and the transaminase level in these patients was normalized after the causative drugs were tapered or discontinued.

Relapse, Causes of Death, and Survival

One patient (UPN 246) who experienced graft rejection underwent retransplantation from the same donor using a slightly intensified preconditioning treat-

ment, including 4 Gy of TBI [14]. As a result, this patient achieved donor-type engraftment without development of GVHD and remained in CR on day 1438. Four patients had a transplantation-related death: 1 due to fungal infection, 1 due to adenovirus infection, 1 due to TMA after hemorrhagic cystitis, and 1 due to TMA. One patient (UPN 317) could not achieve CR, and 5 patients experienced relapse at a median of 144 days (range, 22–324 days) after transplantation. All of these patients died due to progressive disease. The relapse rate at 3 years was 27.1% for patients in CR/chronic phase (CP) (n = 9) and 29.8% for those in non-CR (n = 17) at the time of transplantation (Table 3). Fifteen patients survived in CR at a median follow-up of 664 days (range, 69–1697 days). The event-free survival at 3 years was 55.0% (64.8% for patients in CR/CP and 49.9% for those in non-CR at time of transplantation).

DISCUSSION

One objective of the present study was to determine the donor engraftment rate using a non–T-cell–depleted NST regimen from HLA-haploidentical donors. The conditioning treatment comprising fludarabine, busulfan, and ATG was sufficiently immunosuppressive to achieve the engraftment of HLA-haploidentical donor grafts: 25 of the 26 recipients (96.2%) achieved donor-type engraftment. The use of PBSCs containing large numbers of T cells (median, 2.54×10^8 $CD3^+$ cells/kg) and natural killer (NK) cells (median, 4.25×10^7 $CD56^+$ cells/kg) should be beneficial. The duration and degree of pancytopenia and engraftment of the transplantation cohort in this study were comparable to those found in HLA-matched NST recipients by Slavin et al. [10]. The median duration of ANC $< 0.1 \times 10^9$/L was just 2 days, resulting in a low incidence of severe infections during the neutropenic period (with only 1 patient developing a fatal fungal infection). Seventeen of the 26 patients had 4/6 or 5/6 matches in the HVG direction, which may have contributed to the rapid hematopoietic recovery.

The analysis of donor/recipient chimerism status during the first 2 weeks after transplantation revealed that the very rapid engraftment of donor T cells, with almost 100% donor cell components on day 7. Of note, neutrophils were almost completely of host origin on day 7 and rapidly converted from host to donor origin over the following week (day 7–day 14) (Figure 3). These findings suggest that the achievement of full donor T-cell chimerism drove the elimination of host hematopoietic cells, coupled with the increase of donor hematopoietic cell components.

Another objective of the present study was to assess the incidence and severity of acute GVHD. Less-intensive regimens should be associated with lower toxicity, less release of inflammatory cytokines, and potentially less GVHD. However, Slavin et al. [11], in a study of NST from matched sibling donors using conditioning with fludarabine, busulfan and ATG, reported severe GVHD (≥ grade II) in 38.5% of recipients. Other investigators reported that 28.3% of HLA-matched NST recipients developed severe GVHD using a similar NST protocol [27]. In addition, in the present study, the risk of acute GVHD should have increased markedly due to the use of an extensively HLA-mismatched donor and the rapid achievement of full donor chimerism after SCT. However, among the 25 patients who achieved donor type engraftment, only 5 (20%) developed severe GVHD (grade II). Considering that 2 of these patients (UPN 266 and UPN 315) developed GVHD after the rapid tapering off of steroids and DLI for lymphoproliferative disease or severe hemorrhagic cystitis, severe GVHD can be considered mostly suppressed.

The reasons behind the lower incidence of severe GVHD in the transplantation cohort in this study remain to be determined. To intensify GVHD prophylaxis, we chose a combination of FK506 and mPSL. Steroids are effective for most patients with acute GVHD and thus also should be useful in the prophylaxis of GVHD. However, their role in the prevention of GVHD is not well established in HLA-identical sibling BMT. The addition of steroids to CSP+MTX significantly reduced the incidence of acute GVHD in the studies of Ruutu et al. [28] and Chao et al. [29], but not in those of Atkinson et al. [30] and Storb et al. [31].

In GVHD prophylaxis containing steroids, the speed of steroid tapering may affect the incidence of severe GVHD. For instance, in the study of Storb et al. [31], in which no beneficial effect of steroids on the occurrence of acute GVHD was observed, PSL was initiated on the day of transplantation at 1 mg/kg per day; the dose was halved at 3 weeks, and PSL was discontinued 35 days posttransplantation. In that study, acute GVHD occurred mainly after day 22. In contrast, a study reported by Chao et al. [29], in which steroids were tapered slowly over 180 days, found only a 9% incidence of severe GVHD (II–IV) in patients receiving CSP + MTX + steroid prophylaxis. Furthermore, our previous study of 20 consecutive cases of unrelated bone marrow transplantation, including 3 cases of 2 allele-mismatched donors and 9 cases of 1 allele-mismatched donor, using GVHD prophylaxis consisting of FK506, MTX, and mPSL and a slowly tapered regimen of steroids, found complete suppression of acute GVHD grade II–IV [20]. These results strongly suggest that the speed of steroid tapering is important in transplantation involving steroid-containing GVHD prophylaxis. In addition, steroids may be more useful in HLA-mismatched allogeneic SCT

than in HLA-matched allogeneic SCT. Even the study of Storb et al. [31] found that in HLA-nonidentical BMT recipients, adding PSL significantly decreased the incidence of acute GVHD (all 11 patients not given PSL and 6 of 12 patients given PSL developed acute GVHD; $P = .01$), although the reason for this was not discussed. Furthermore, in our previous study of HLA-haploidentical NST [12], in which patients received the same conditioning regimen as in the present study (ie, combination of fludarabine, busulfan, and ATG, along with non–steroid-containing GVHD prophylaxis, such as CSP or FK506 monotherapy), 67% of the patients developed severe acute GVHD. Based on these findings, we consider that adding steroids to GVHD prophylaxis contributed to the suppression of severe GVHD. Although Chao et al. [29] recommended steroid administration from day 7, we started the agent on day 0, because donor T cells have been reported to proliferate much more rapidly and aggressively in major histocompatibility complex (MHC)-mismatched transplantation settings than in MHC-matched transplantation settings [32].

Regarding the low incidence of severe GVHD, another point to emphasize is the importance of early therapeutic intervention for GVH response. The typical pattern of GVHD during the engraftment period is as follows. The patient presents with low-grade fever under a negative test (or low-grade positive) for C-reactive protein at an early transplantation stage (day 5–7), when neutrophil counts are still decreasing. The low-grade fever soon changes to a high-grade spiking fever. With the persistence of fever for approximately 3 days, skin eruptions becomes evident, which rapidly progress to generalized cutaneous lesions (erythroderma) with or without the development of GVHD symptoms in other target organs (grades II–IV). Starting mPSL 2 mg/kg immediately after the appearance of skin eruptions usually fails to suppress the progression of GVHD. Even if GVHD symptoms can be successfully controlled, life-threatening GVHD-associated complications, such as TMA, often occur later. Thus, we consider it important to initiate the therapeutic intervention during the febrile period before GVHD becomes evident. In the present study, the 2 patients treated in this manner avoided clinical GVHD. Once a patient manages to weather the storm of the GVH reaction during the engraftment phase, the magnitude of allogeneic response appears to be rapidly reduced thereafter, in part because massive activation-induced cell death occurs in MHC-mismatched transplantation settings [33,34]. In the present protocol, because the neutropenic period was very short (as discussed in Results), fever due to bacterial or fungal infection occurred only rarely. However, the emergence of any sign or symptom suggesting infection warrants concurrent treatment for infection.

Rapid achievement of donor-type engraftment after transplantation must have an advantage in terms of exerting antitumor effects by the allogeneic immunologic response [35]. All 4 patients with non-Hodgkin's lymphoma who had a relapse after receiving autologous SCT achieved CR and have remained in CR at a median follow-up of 689 days (range, 355–1159 days), strongly suggesting the presence of a powerful graft versus leukemia (GVL) effect in this transplantation modality (although this study involved only a small number of patients). Regarding the GVL effects exerted by HLA-haploidentical grafts, several studies have shown that NK cell alloreactivity is associated with a significant GVL effect, particularly in patients with acute myeloid leukemia [36,37]. We could not assess the GVL effects by NK cells in the present study, because only 5 patients had a disparity in the killer inhibitory receptor epitopes in the GVH direction. In contrast, the recovery of $CD4^+$ T cells appeared to be delayed after transplantation, possibly related to long-term administration of immunosuppressive agents (even at low doses). Although CMV antigenemia was observed in 21 of 26 patients (80.8%), the occurrence of CMV disease could be effectively prevented by early and appropriate administration of ganciclovir or foscarnet. However, this delayed recovery of $CD4^+$ T cells may have led to several types of viral infection. Three patients developed life-threatening viral infections: adenoviral pneumonia (UPN 218), human herpesvirus-6 encephalitis (UPN 286), and lymphoproliferative disease (UPN 266). Consequently, viral infections should be carefully monitored for in this transplantation modality.

In conclusion, in non–T-cell–depleted HLA-haploidentical (2–3 antigen-mismatched) SCT using nonmyeloablative conditioning comprising fludarabine, busulfan, and ATG, along with GVHD prophylaxis with FK506 and mPSL (1 mg/kg/day), donor-type engraftment was achieved in 25 of 26 patients (96.2%), and only 5 patients (20%) developed severe acute GVHD. Thus, our findings demonstrate that this transplantation procedure may be feasible for patients who are at high risk and lack available donors and also for those considered ineligible for myeloablative preparative regimens due to advanced age or comorbidities, although our results need to be confirmed in a large-scale study.

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